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POPULATION GENETIC STRUCTURE AND CONSERVATION OF AN ENDANGERED CONIFER, *CATHAYA ARGYROPHYLLA* (PINACEAE)

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Cathaya argyrophylla is an endangered conifer restricted to subtropical mountains of China, with total number of individuals less than 4,000. To assess levels and patterns of genetic diversity of *C. argyrophylla,* eight populations representing four widely separated regions were analyzed for allozyme variation, using 13 enzymes. In comparison with other coniferous species, *C. argyrophylla* possesses a low amount of variation, particularly at the population level (A = 1.38; P = 30.4; H_e = 0.102). By contrast, the level of population differentiation is much higher (F_{ST} = 0.441) compared to other conifers, and significant differentiation occurs both between regions and between populations within a region. Climate, geologic, and fossil data suggest that historical factors are mainly responsible for the unique population genetic structure in *C. argyrophylla.* These factors include severe bottleneck and subsequent genetic drift during Quaternary glaciations and habitat deterioration and fragmentation in postglaciation. In addition, reduced gene flow and relatively high rates of inbreeding may be factors that lead to low population variability and marked genetic differentiation among populations. Implications for the development of conservation strategies for this endangered species are discussed on the basis of these findings.

Introduction

Intraspecific levels of variation are a necessary prerequisite for any future adaptive change or evolution and have profound implications for species conservation (Millar and Libby 1991; Schaal et al. 1991). In recent decades, allozyme electrophoresis has been a dominant technique for examining genetic variation and has provided the most abundant source of information concerning genetic diversity in plant species (Hamrick and Godt 1989; Schaal et al. 1991; Hamrick et al. 1992). Recent studies utilizing pooled isozyme data from the literature (Hamrick and Godt 1989; Hamrick et al. 1992) indicate that one of the generalizations concerning the relationship between genetic diversity and the characteristics of species is that long-lived, outcrossing, wind-pollinated, late-successional species have higher levels of allozyme variation within populations and less variation among populations than do species with other combinations of traits. As a group having life-history traits associated with high levels of genetic variation, gymnosperms (mostly conifers) maintain relatively high levels of genetic variability and display little genetic differentiation among populations (mean P = 71.1; H_e = 0.169; G_{ST} = 0.073; Hamrick et al. 1992). Nevertheless, as the number of reliable studies has increased, it has become obvious that both genetic diversity and population structure vary considerably among conifer species (Ledig 1986; El-Kassaby 1991; Hamrick et al. 1992). Taking the genus *Pinus* as an example, genetic diversity within species ranges from as low as H_e = 0.0 for *P. resinosa* (Fowler and Morris 1977; Simon et al. 1986) to as high as H_e = 0.327 for *P. longaeva* (Hiebert and Hamrick 1983). Likewise, G_{ST} values (measuring population differentiation) cover a large range with some species having low variation among populations (G_{ST} < 0.03 for *P. rigida,* Guries and Ledig 1982; *P. banksiana,* Dancik and Yeh 1983; *P. longaeva,* Hiebert and Hamrick 1983) to a few having approximately one-third of their genetic diversity among populations (G_{ST} = 0.300 for *P. halepensis,* Schiller et al. 1986; G_{ST} = 0.337 for *P. merkusii,* Szmidt et al. 1996).

Although conifers are the most extensively investigated plant group in allozyme surveys, most of the species studied are those having widespread, nearly continuous geographic distributions. Relatively few studies have examined patterns of genetic variation in endemic and rare conifers (Copes 1981; Ledig and Conkle 1983; Millar and Libby 1991; Szmidt et al. 1996). We report here the results of a preliminary survey of electrophoretic variation in *Cathaya argyrophylla* Chun et Kuang, an extremely endangered conifer that occurs in widely separated subtropic mountains in China. Because many aspects of the general history and biology of the species have been investigated (Wang 1990; Fu 1992; Xie 1996), it appears to be an attractive organism on which to conduct empirical studies exploring the influence of natural history and geographic isolation on levels of genetic variability within and among populations. In addition, the results of the study may also allow initial strategies to be developed for its conservation.

The specific goals of this study are (1) to determine the amount of genetic diversity within and among populations; (2) to examine how the distribution of genetic variation within and among populations varies over spatial scales; (3) to seek historical, life-history, and/or environmental factors that might explain the patterns and levels of genetic variation observed; and (4) to

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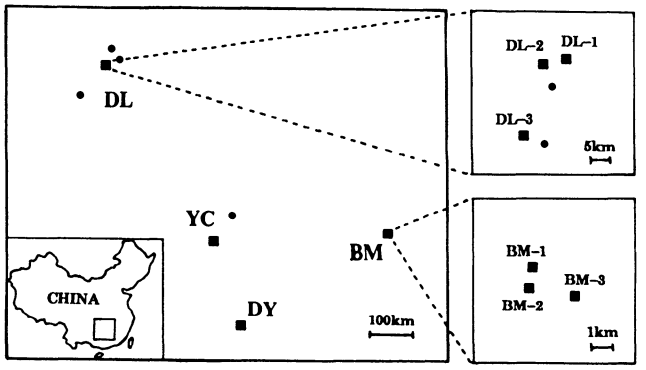

Fig. 1 Distribution map of *Cathaya argyrophylla.* Populations were assigned to four geographic regions (*BM, DL, DY,* and *YC*) as described in the text. The squares indicate the locations of populations sampled in this study.

apply this information to develop recommendations for the recovery and management of this endangered conifer.

Material and Methods

Study Species and Populations

Cathaya argyrophylla, like most conifers, is a monoecious, wind-pollinated, and predominantly outcrossing species. Currently, it is a member of the needle-leaved and broad-leaved mixed forest in middle-elevation mountains of the subtropical China, where rainfall is abundant and the climate is cool in the summer and cold in the winter (Wang 1990; Fu 1992). It was discovered in 1955 in Huaping, Longsheng County, Guangxi, and was placed in a monotypic genus in the family Pinaceae on the basis of its taxonomic distinctness (Chun and Kuang 1952; Fu 1992). At present, this species consists of fewer than 4,000 individuals and is confined to four widely separated mountainous regions, i.e., the Daloushan mountains (DL), the Bamianshan mountains (BM), the Yuechengling mountains (YC), and the Dayaoshan mountains (DY) (fig. 1), with population sizes ranging from one to several dozens (Xie 1996). In addition to its limited number of individuals and small population sizes, the species is characterized by unusually low fertility and very low survival and growth rates (Wang 1990; Xie 1996; Z.-K. Chen, unpublished data). On average, for example, there were only 4.3 seeds per cone, with as many as 12.2% of cones producing no seed, though the cone production was very low in nature and the percent of seed germination in the field was merely 21% (Xie 1996). Furthermore, on the basis of extensive examination of its reproductive development in natural condition, 60%–90% ovule abortion, and ca. 80% embryonic mortality were recently found (Z.-K. Chen, unpublished data). In particular, much evidence suggests that the existing populations of *C. argyrophylla* in various communities are declining and at risk of being replaced by fast-growing, broad-leaved trees (Fu 1992; Xie 1996). Accordingly, *C. argyrophylla* is currently listed as one of the eight most endangered plant species in China (Wang 1990; Fu 1992).

During three consecutive autumns from 1992 to 1994, we have made extensive collections of cones throughout the range of *C. argyrophylla.* Because of very low cone and seed production, as well as difficult access, only one or two individual trees were sampled from some populations, which were excluded from our allozyme analysis. Also, the sample sizes in a number of populations were relatively small in spite of most cone-bearing trees being sampled. The present study was based on 101 trees sampled in eight populations representing the four regions, including all three populations in region BM, three populations in region DL, and one each from regions DY and YC. The straight-line distances between the sampled populations ranged from less than 1 km to ca. 700 km (fig. 1). The location and sample size of each population are shown in figure 1 and table 2.

Cones were collected from individual trees except for population YC, in which a bulk collection was obtained (in excess of 20 individuals) in Huaping, Longsheng. Wind-pollinated seeds from collected cones were extracted by hand, stored at 0°C, and maintained by individual mother-tree identity throughout processing and storage.

Electrophoresis

Seeds were imbibed with distilled water for approximately 10 days at room temperature before each megagametophyte was separated cleanly from its seed coat and embryo. For estimating parental genotypes, a minimum of 10 megagametophytes per parent tree were assayed. This approach will correctly identify a heterozygous locus with probability $1 - 0.5^9 = 0.998$. For population YC, where seeds were obtained from a mixed collection, 120 megagametophytes were analyzed to compute allele frequencies of the population. Crude extracts were obtained by crushing single magagametophytes in one drop of 0.1 M Tris-HCl, pH 7.5 buffer with 0.1% v/v 2-mercaptoethanol. The homogenates were absorbed on paper wicks, and electrophoresis was performed with 12% starch gels.

Four buffer systems were used for separating enzymes. Of them, three followed those of Soltis et al. (1983). System 6 was used to resolve aspartate aminotransferase (AAT), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and triose-phosphate isomerase (TPI). System 9 was used to resolve malate dehydrogenase (MDH), malic enzyme (ME) and 6-phosphogluconate dehydrogenase (6PGD). System 11 was used to resolve AAT (for *Aat-1*), alcohol dehydrogenase (ADH), diaphorase (DIA), fructose-bisphosphate aldolase (FBA), and isocitrate dehydrogenase (IDH). Another two enzymes, leucine aminopeptedase (LAP) and shikimate dehydrogenase (SKD), were resolved on the buffer system of Clayton and Tretiak (1972). Staining procedures for all enzymes followed the methods of Soltis et al. (1983) and Wendel and Weeden (1989). When more than one locus were detected for an enzyme, loci were numbered sequentially, with the most anodally migrating isozyme designated 1. Different alleles at a locus were identified using letters, with the most anodal as *a,* the next as *b,* and so on.

Data Analysis

Electrophoretic data were entered as genotypes or gene frequency (for population YC) and analyzed using the computer program BIOSYS-1 version 1.7 (Swofford and Selander 1989) for IBM-PC. Genetic diversity statistics, percentage of polymorphic loci (P), mean number of alleles per locus (A), observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated for each population. Wright's (1965) fixation index (F) was also computed for all populations and polymorphic loci to detect heterozygosity deficiency or excess. In addition, population and regional differentiation was investigated using Wright's (1978) F-statistics, which are related by $(1 - F_{IS}) \times (1 - F_{ST}) = (1 -$

F_{IT}), where F_{IT} is the total deviation from expected frequencies under Hardy-Weinberg equilibrium, F_{ST} is the deviation due to population subdivision, and F_{IS} is the deviation within population subdivisions. The F_{ST} for multiple alleles was obtained as a weighted average of F_{ST} for all alleles and, therefore, is equivalent to Nei's (1977) G_{ST}, which was used to estimate the proportion of genetic diversity among populations.

A cluster analysis using UPGMA and Nei's (1978) unbiased genetic distance was also performed. Associations between genetic distance and linear geographic distance were examined using a correlation analysis. An estimate of gene flow among populations (N_m) can be computed from the multilocus value of F_{ST} as $F_{ST} = 1/(4N_{ma} + 1)$, where $a = (n/[n - 1])^2$, n is the number of populations, and N_m is the number of migrants per generation (Nei 1973).

Results

In our survey, 25 allozyme loci were resolved with sufficient consistency and clarity for 13 enzyme systems. Of them, 13 loci (*Aat-3, Adh, Dia-1, Dia-2, Dia-3, Fba-2, Idh-2, Mdh-1, Pgm-1, Pgm-2, Tpi-1, Tpi-2,* and *Tpi-3*) were monomorphic, with all individuals from all populations possessing a single enzyme band with identical mobility for each locus. The remaining 12 loci (48%) were polymorphic (0.99 criterion) in at least one of the eight populations surveyed. *Aat-1, Fba-2, Idh-1, Lap-1, Pgd-2, Pgi-1,* and *Skd* each had two alleles; *Aat-2, Lap-2, Mdh-2,* and *Me* each had three alleles; *Pgi-2* had four alleles. Allele frequencies at the polymorphic loci for each population are given in table 1. Allele frequencies varied greatly among the four regions and the eight populations. In addition to the dissimilar frequencies of shared alleles at many loci, the differences were especially marked by the presence and absence of unique alleles (e.g., *Idh-1c, Pgi-1a,* and *Skd-c* presented only in population YC; *Mdh-2a* fixed in BM-3) (table 1). On the regional scale, *Pgd-2a* was the most common allele or fixed at the populations from regions BM and YC, whereas *Pgd-2b* was the most common alleles or fixed at the populations from regions DL and DY (table 1).

Genetic Diversity

Low levels of genetic variation at population level were observed (table 2). These standard measures of genetic diversity varied among populations. Population YC had the highest values for these genetic variability statistics ($A = 1.60$; $P = 40.0$; and $H_e = 0.141$), whereas the remaining seven populations from the other three regions had uniformly the lower values. The average values of A, P, H_o, and H_e for all the populations were 1.38, 30.4, 0.097, and 0.102, respectively. By comparison, relatively higher variability was found at species level, with A, P, and H_e being 1.80, 48.0, and 0.159, respectively (table 2).

To test the conformance of genotype frequencies to Hardy-Weinberg expectations, the fixation index, F, was calculated within each population for each polymorphic locus. In 47 comparisons of observed and expected genotypic distributions (not shown), 37 (78.7%) exhibited negative fixation and the remaining 10 positive fixation, but none of them was statistically significantly different from 0. These results imply that these populations approach random mating. At population level (table 2), a slight deficiency of heterozygosity was detected in populations BM-1 and DL-3. In contrast, excess of heterozygosity was observed in the other four populations, particularly in population DY, with fixation index of -0.234. The mean F_{IS} value of -0.099 (table 3) further indicates a slight excess of heterozygotes in these eight populations.

Distribution of Genetic Variation among Populations and Regions

For the populations we examined, substantial population differentiation was found for all polymorphic loci, with F_{ST} values ranging from 0.094 at *Idh-1* to 0.790 at *Pgd-2* (table 3). Averaged over all loci, 44.1% of the total diversity was apportioned among populations. The gene flow estimate was very low ($N_m = 0.233$), which corroborated the finding that rather high genetic differentiation existed among populations. In particular, very high population differentiation was observed among populations within regions. The highest G_{ST} value within a region was found among the three populations within region BM (0.301), even though they were all found 1–2 km apart and could easily be considered subpopulations (fig. 1).

In Nei's (1978) unbiased genetic distance calculations, genetic distances between populations ranged from 0.004 between population DL-2 and DL-3 to 0.186 between BM-2 and DL-1, with a mean of all pairwise comparisons of 0.078. The mean distance between regions was 0.071, which was not significantly different from the average between populations within regions (0.060) (fig. 2). The cluster phenogram constructed using genetic distances between populations revealed that populations DL-1 and BM-3 were the most genetically differentiated and that populations within each region did not cluster together before forming a cluster with any population of other regions (fig. 2), indicating that there is distinct differentiation within regions (e.g., the DL and BM regions). As a result, there is no trend that genetic distance increases with geographic separation. The regression tests further demonstrated that no correlation was found between genetic distance value and the linear geographic distance.

Discussion

Genetic Structure of Populations

Our electrophoretic survey of the eight populations of *Cathaya argyrophylla* indicates that this endangered conifer displays low level of genetic variability, particularly at population level, compared to plants with similar life-history characteristics (Yeh and O'Malley 1980; Dancik and Yeh 1983; Hiebert and Hamrick 1983; Ledig 1986; and see table 4 for comparisons). It is noteworthy that *C. argyrophylla* shows very high level of interpopulation

Table 1 Allele Frequencies at the Polymorphic Loci in the Eight Populations of *Cathaya argyrophylla*

	Population							
Locus	BM-1	BM-2	BM-3	DL-1	DL-2	DL-3	DY	YC
Aat-1:								
a	0.000	0.000	0.000	0.611	0.265	0.167	0.071	0.125
b	1.000	1.000	1.000	0.389	0.735	0.833	0.929	0.875
Aat-2:								
a	0.000	0.000	0.000	0.800	0.088	0.000	0.045	0.083
b	0.917	0.955	0.857	0.200	0.824	1.000	0.955	0.917
c	0.083	0.045	0.143	0.000	0.088	0.000	0.000	0.000
Fba-1:								
a	0.222	0.088	0.063	0.000	0.000	0.250	0.000	0.000
b	0.778	0.912	0.938	1.000	1.000	0.750	1.000	1.000
Idh-1:								
a	0.250	0.139	0.125	0.000	0.133	0.000	0.000	0.050
b	0.750	0.861	0.875	1.000	0.867	1.000	1.000	0.925
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025
Lap-1:								
a	0.143	0.500	0.214	0.778	0.382	0.500	0.000	0.325
b	0.857	0.500	0.786	0.222	0.618	0.500	1.000	0.675
Lap-2:								
a	0.000	0.000	0.000	0.056	0.118	0.000	0.000	0.000
b	1.000	1.000	1.000	0.944	0.647	0.833	1.000	0.775
c	0.000	0.000	0.000	0.000	0.235	0.167	0.000	0.225
Mdh-2:								
a	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
b	0.688	0.833	0.000	0.000	1.000	0.833	0.083	0.000
c	0.313	0.167	0.000	1.000	0.000	0.167	0.917	1.000
Me:								
a	0.222	0.528	0.143	0.750	0.353	0.000	0.083	0.389
b	0.778	0.472	0.857	0.188	0.647	1.000	0.833	0.139
c	0.000	0.000	0.000	0.063	0.000	0.000	0.083	0.472
Pgd-2:								
a	1.000	1.000	1.000	0.111	0.088	0.000	0.250	0.750
b	0.000	0.000	0.000	0.889	0.912	1.000	0.750	0.250
Pgi-1:								
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.875
Pgi-2:								
a	0.000	0.000	0.000	0.444	0.118	0.000	0.000	0.200
b	0.125	0.333	0.857	0.000	0.000	0.000	0.000	0.025
c	0.000	0.028	0.000	0.444	0.029	0.000	0.318	0.125
d	0.875	0.639	0.143	0.111	0.853	1.000	0.682	0.650
Skd:								
a	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.146
b	1.000	1.000	1.000	1.000	1.000	1.000	0.750	0.750
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.104

Note. Population numbers are the same as in figure 1.

Table 2 Genetic Variability and Fixation Indices at 25 Loci for the Eight Populations of *Cathaya argyrophylla*

Population	*N*	*A*	*P*	H_o	H_e	*F*
BM-1	10	1.3	28.0	0.081	0.090	0.100
BM-2	18	1.3	28.0	0.097	0.092	−0.054
BM-3	9	1.2	24.0	0.066	0.060	−0.100
DL-1	11	1.4	28.0	0.103	0.103	0.000
DL-2	17	1.4	32.0	0.124	0.115	−0.078
DL-3	4	1.2	20.0	0.073	0.084	0.131
DY	12	1.3	28.0	0.095	0.077	−0.234
YC	20	1.6	40.0	...	0.141	...
Mean		1.38	30.4	0.097	0.102	...
Species ..	101	1.80	48.0	0.076	0.159	...

Note. *N* is number of trees sampled.

differentiation (G_{ST} = 0.441), which appears so far the most variable conifers in terms of variation among populations (see El-Kassaby 1991, for review). As shown in table 4, the genetic diversity within species and populations of *C. argyrophylla* are 30%–50% lower than the average of more than 100 gymnosperms, and *C. argyrophylla* possesses five times higher genetic variation among populations than does the average of these gymnosperms. Our recent RAPD survey further corroborated these findings (Wang et al. 1997). Similar levels and patterns of allozyme variation have recently been found for other Asian *Pinus* species also (Son et al. 1989; Szmidt et al. 1996).

Our examination of climatic, geologic, and fossil data suggests that the unusual population genetic struc-

Table 3 Summary of *F*-Statistics at All Loci of *Cathaya argyrophylla*

Locus	F_{IS}	F_{IT}	F_{ST}
Aat-1	−0.224	0.167	0.319
Aat-2	0.009	0.462	0.457
Fba-1	−0.025	0.098	0.120
Idh-1	−0.217	−0.102	0.094
Lap-1	0.019	0.270	0.256
Lap-2	−0.023	0.149	0.168
Mdh-2	0.036	0.741	0.731
Me	0.072	0.335	0.284
Pdg-2	−0.225	0.743	0.790
Pgi-2	−0.381	0.224	0.438
Skd	−0.33	−0.037	0.222
Mean	−0.099	0.385	0.441

Note. Population YC was excluded from the calculation, because of the bulked sampling.

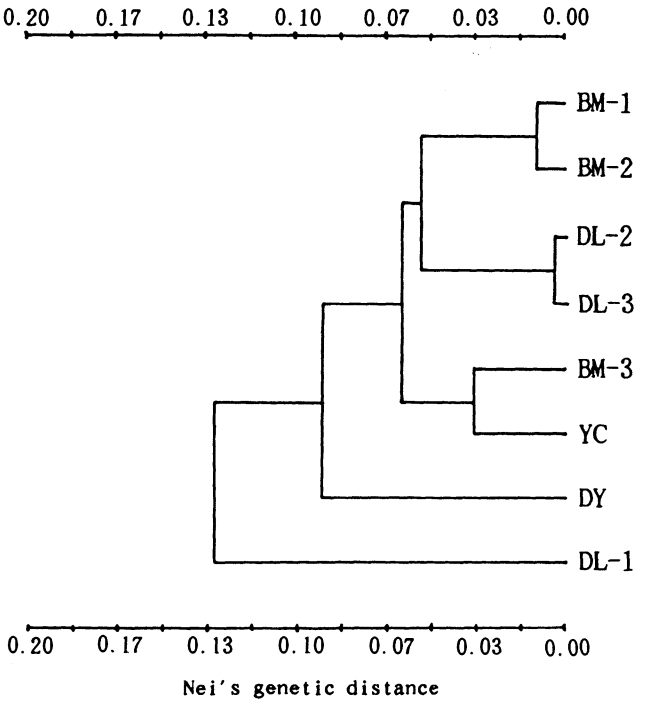

Fig. 2 Cluster phenogram using Nei's genetic distance values for populations of *Cathaya argyrophylla*.

ture of *C. argyrophylla* could be attributed to its evolutionary history. At present, fossils of *C. argyrophylla* are known from the Miocene in Eschweiler of Germany and in eastern Siberia (Florin 1963; Wang 1990). Its pollen has also been found from the Oligocene to Miocene sediments in what is now the southwestern part of France and the early Quaternary in Yunnan Province of China (Wang 1990; Fu 1992). Consequently, the fossil evidence, although scant, may indicate that *C. argyrophylla* was much more widely distributed in Europe and eastern Asia in the Miocene than at present. Thus, it is likely that repeated invasions of glaciers during the Quaternary eliminated this species from the northern part of the Eurasia (Xie 1996). The effect of glaciations on shaping geographic patterns of allozyme variation has been noticed in other conifers such as *P. resinosa* (Fowler and Morris 1977), *Thuja plicata* (Copes 1981), and the rare *P. torreyana* (Ledig and Conkle 1983).

In our case, historical factors might have affected the genetic profiles of *C. argyrophylla* in a number of ways. First, severe bottleneck and subsequent genetic drift are the most likely explanation for the unique genetic structure of the species. Apparently, the geographic range of *C. argyrophylla* was largely shrunk and its populations were greatly reduced in size during cold periods of the Quaternary glaciations (Wang 1990; Xie 1996). Theoretically, reductions in population size create genetic bottlenecks because remaining individuals contain only a small sample of the alleles present in the parental generation (Ellstrand and Elam 1993). In this connection, *C. argyrophylla* has been genetically depauperate through the bottleneck, when it was forced into small and isolated populations. The much higher value of F_{ST} relative to the mean of other gymnosperms (table 4), as well as no correlation between genetic distance and geographic distance (figs. 1 and 2), support the notion that genetic drift has taken place in this species. Another piece of evidence is that some alleles at many loci are randomly fixed in geographically distant populations. For example, at locus *Fba-1,* allele b is fixed in the four populations belonging to the three widely separated regions (DL-1, DL-2, DY, and YC), and at locus *Lap-2,* allele b is fixed in all populations of the BM region and geographically the most distant population DY. Similar situations are also found at loci *Idh-1, Mdh-2,* and *Skd* (fig. 1; table 1).

The second factor is likely inbreeding. It is well

Table 4 Mean Genetic Diversity and Population Differentiation for *Cathaya argyrophylla* as Compared to Other Gymnosperm Species[a]

Species	P_s	P_p	A_s	A_p	H_{es}	H_{ep}	G_{ST}
C. argyrophylla	48.0	30.4	1.80	1.38	0.159	0.102	0.441
Gymnosperms (89–121)[b] ...	71.1	53.4	2.38	1.83	0.169	0.151	0.073
All plant species (662)[b]	51.3	34.6	1.97	1.52	0.150	0.113	0.228

[a] P_s and P_p are the percent polymorphic loci within species and populations, respectively; A_s and A_p, the mean number of alleles per locus within species and populations, respectively; H_{es} and H_{ep}, the expected heterozygosity within species and populations, respectively; G_{ST}, the coefficient of gene differentiation.

[b] Data from Hamrick et al. (1992). Numbers in parentheses are the number of taxa reviewed.

recognized that two genetic consequences of small population size are increased genetic drift and inbreeding (Barrett and Kohn 1991; Ellstrand and Elam 1993). Much evidence indicates that conifers often express severe inbreeding depression in many life stages because of embryonic mortality (Savolainen 1994). Considering the high mortality and depression in *C. argyrophylla,* it is expected that the species has relatively high rate of inbreeding. Nevertheless, as revealed by our allozyme data, *C. argyrophylla* populations are largely panmictic or even showed minor excess of heterozygotes (tables 2 and 3). No indication of inbreeding in the present study may have resulted from the materials we used in the analysis. As noted by Muona (1989), a low proportion of selfs among mature seeds exists in most species. For example, Eguiarte et al. (1992) found in *Astrocaryum mexicanum* (a tropical palm) that the mean fixation index at seed stage was −0.2 and −0.4 at adult stage. Similarly, in *Pinus sylvestris,* there were some selfs at the seed stage but no evidence of inbreeding was present in adult populations (Savolainen 1994). Consequently, the information relevant to mating system in our case may not accurately reflect the true profile of *C. argyrophylla,* because the genotype data are only for those trees that have survived to mature. In addition, it is reported that the pollination and mating system of *C. argyrophylla* seem little different from other conifers, and the primary seed dispersal is through gravity and wind, with secondary dispersal facilitated by squirrels (Wang 1990; Xie 1996). However, the squirrel dispersal strategy did not work effectively enough, partly because of very low rates of seed germination and seedling survival (Xie 1996), and the pollen flow appears to be blocked in some ways, because even the nearest populations within the same region have entirely different dominant alleles or fixed alleles, e.g., the BM region (table 1). Consequently, reduced gene flow may be a factor that has led to the marked genetic differentiation among populations in *C. argyrophylla.*

Finally, it is worthwhile mentioning that although great efforts have been made, the sample sizes per population in the present study were relatively small, which may lead to biased estimates toward some statistics to a certain extent. Detailed studies of the reproductive biology, population demography, and mating system of this species are currently under way and should provide an insight into the population genetics and evolution of this endangered species.

Implications for Conservation

Knowledge of levels and distribution of genetic variation is a prerequisite for the establishment of effective and efficient conservation practices. As one of the most endangered plant species in China, *Cathaya argyrophylla* has been given high priority for protection since it was discovered three decades ago. The unique population genetic structure revealed by our electrophoretic study is instructive to implementing efficient and practical conservation programs.

First, given the limited number of individuals and populations, it is necessary to protect all the existing populations in situ in order to preserve as much genetic variation as possible, particularly populations such as population YC, which showed the highest variability and harbored most rare alleles. Second, because the genetic diversity of in situ conserved populations should be dynamically maintained and evolved with the changing environments, habitat protection, in the long term, is thus more important for preventing the species from further loss of genetic variation and decrease of population size. This is especially true, considering that habitats suitable for this species are reported to be in the process of deterioration and fragmentation (Fu 1992; Xie 1996). On the other hand, with knowledge of genetic architecture available, an appropriate strategy for sampling and propagation could be easily formulated when ex situ conservation is required. Finally, as we postulated above, if inbreeding depression takes place in *C. argyrophylla,* it is likely to restore and enrich genetically the threatened or declining populations by employing traditional breeding programs, e.g., controlled crossing between genetically distinct populations. No genetic information on the adaptive characters such as morphological or physiological traits is available for *C. argyrophylla.* Without any question, such information is crucial to a thorough understanding of the genetic architecture and to making appropriate management decisions for the conservation of *C. argyrophylla.*

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